

PCR (Polymerase Chain Reaction)

Requirements:

- Takara PrimeSTAR® Max DNA Polymerase
- DNA template
- Primers (synthesized by Sangon Biotech®)
- Nuclease-free 0.2mL PCR tubes
- PCR instrument

Protocol:

1. The choice of reaction system:

Components	Volume
DNA template	2μL
Forward Primer	1μL
Reverse Primer	1μL
PrimeSTAR® Max DNA Polymerase	20μL
Sterile deionized water	16μL

2. The choice of PCR program:

Step	Temperature	Duration	Loops
Preheat	97°C	—	—
1	94°C	5 minutes	
2	94°C	30 seconds	30 loops
3	x	30 seconds	
4	72°C	1 minute	
5	72°C	10 minutes	—
6	15°C	10 minutes	

Note: x is the annealing temperature of the reaction, usually 5°C to 10°C lower than T_m of the primer.

The calculation of T_m: $T_m = 4(G+C) + 2(A+T)$